PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference R 41846	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/09591	International filing date *(day/month/year)* 29.08.2003	Priority date *(day/month/year)* 13.09.2002
International Patent Classification (IPC) or both national classification and IPC C12N5/06		
Applicant FORSCHUNGSINSTITUT FÜR KREBSKRANKE KINDER, et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13.04.2004	08.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Lanzrein, M Telephone No. +49 89 2399-7358

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09591

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-23	as originally filed

Claims, Numbers

1-9	as originally filed
10-19	received on 13.04.2004 with letter of 13.04.2004

Drawings, Sheets

1/9-9/9	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

   see separate sheet

6. Additional observations, if necessary:

   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-9 (only IA)

   because:

   ☒ the said international application, or the said claims Nos. 1-9 (only IA) relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/ or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-9
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	-

No: Claims

2. Citations and explanations

see separate sheet

Re Item I

Basis of the report

1. **Added Subject-Matter**

The amendments filed with the letter dated 13. April 2004 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: Claims 10-19 relate to a composition containing LPS and IFN-γ as well as the use of said composition and a kit comprising LPS and IFN-γ.
The compounds LPS and IFN-γ were, however, only disclosed in conjunction with their application on DC's to trigger IL-12 release and have never been disclosed as composition "as such". For example p. 3, 4th paragraph refers to the release of IL-12 from DC by *exposure* to LPS and IFN-γ.
The composition as such is, however, a much broader concept, which is not limited to the use in triggering IL-12 due to the fact that claim 10 is a product claim and is therefore not limited by "for triggering IL-12 release".

Moreover, a kit comprising the said compounds has not been mentioned in the originally filed documents.

Claims 10-19 are therefore not subject to the international preliminary examination.

2. **Medical Use**

Claims 1-9 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: FELZMANN THOMAS ET AL: 'Functional maturation of dendritic cells by exposure to CD40L transgenic tumor cells, fibroblasts or keratinocytes' CANCER LETTERS, vol. 168, no. 2, 26 July 2001 (2001-07-26), pages 145-154, ISSN: 0304-3835

D2: RIESER CLAUDIA ET AL: 'Mature dendritic cells induce T-helper type-1-dominant immune responses in patients with metastatic renal cell carcinoma' UROLOGIA INTERNATIONALIS, vol. 63, no. 3, 1999, pages 151-159, ISSN: 0042-1138

D3: FELZMANN THOMAS ET AL: 'Xenogenization by tetanus toxoid loading into lymphoblastoid cell lines and primary human tumor cells mediated by polycations and liposomes' CANCER LETTERS, vol. 161, no. 2, 20 December 2000 (2000-12-20), pages 241-250, ISSN: 0304-3835

D4: BANCHEREAU J & STEINMAN R M: "Dendritic cells and the control of immunity" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 392, no. 6673, 19 March 1998 (1998-03-19), pages 245-252, XP002134557 ISSN: 0028-0836

D5: GITLITZ B J ET AL: "Dendritic cell-based immunotherapy of renal cell carcinoma." CURRENT UROLOGY REPORTS. UNITED STATES FEB 2001, vol. 2, no. 1, February 2001 (2001-02), pages 46-52, XP009022495 ISSN: 1527-2737

2. The present application concerns the use of dendritic cells loaded with tumour antigens in immunotherapy of cancer. The DC's were matured by treatment with LPS and interferon-gamma and are active in releasing IL-12.
The DC's may be additionally charged with tetanus toxoid as adjuvant and keyhole limpet haemocyanin (KLH) may be used as tracer antigen.

3. **Novelty** (Art. 33 (2) PCT)

The use of DC's loaded with tumor antigens for cancer immunotherapy is well known in the art (reviewed in D4 and D5).
The present application discloses the use of DC's which release IL-12 due to treatment with lipopolysaccharide (LPS) and intereferon-gamma (IFN-γ).
D1 teaches methods for maturation of DC's by exposure to CD40L or LPS in conjunction with IFN-γ (p. 147, 4. §; Fig. 3), whereby maturation was monitored by measuring IL-12 secretion (p. 148, 1. §; Fig. 4). The DC's are produced for the purpose of anti-tumor immunotherapy (see e.g. abstract), however, the use of a tumor antigen is not explicitly disclosed.
Due to the fact that the cells in D1 are not loaded with a tumor antigen, the subject-matter of claims 1-9 is novel over the cited prior art.

4. **Inventive Step** (Art. 33 (3) PCT)

The subject-matter of claims 1-9 is considered inventive for the following reasons:
D1 as the closest prior art document discloses methods for maturation of DC's for tumor immunotherapy. In fact, D1 favours maturation of DC's by exposure to cells expressing CD40L, which leads to much greater IL-12 release (Fig. 4) and expansion (Fig. 5) than LPS stimulation. Thus, starting from D1, the skilled person would not have used LPS for the maturation process, as is proposed in the present application. Therefore, D1 teaches away from the subject-matter of the present claims. The other documents do not propose the combination of LPS and IFN-gamma for maturation of DC's for the purpose of tumor-immunotherapy.

5. **Clarity** (Art. 6 PCT)

It appears the present claims 1-9 are broadly referring to DC's which release IL-12 "due to treatment with lipopolysaccharide (LPS) and interferon-gamma". No time-frame nor any concentration values are specified for the treatment. Moreover, the amount of IL-12 release is not defined. The claims thus lack sufficient technical characterization in order to clearly define the scope of the protection which is sought (Art. 6 PCT).

New claims:

1. Use of active dendritic cells (DCs) releasing interleukin 12 (IL-12) which are loaded with an antigen against a specific tumor and, due to the treatment with lipopolysaccaride (LPS) and interferon-gamma (IFN-γ), release IL-12, for the preparation of a medicament for treating a patient having said specific tumor.

2. Use according to claim 1, characterised in that said treatments is performed after bone marrow transplantation.

3. Use according to claim 1 or 2, characterised in that said specific tumor is an advanced malignancy.

4. Use according to any one of claims 1 to 3, characterised in that in said DCs are DCs having been taken from the patient having said specific tumor or from the bone marrow donor.

5. Use according to any one of claims 1 to 4, characterised in that the DCs have been loaded with an antigen from a tumor cell from said patient having said specific tumor.

6. Use according to any one of claims 1 to 5, characterised in that the DCs are additionally charged with a tracer antigen.

7. Use according to claim 6, characterised in that said tracer antigen is keyhole limpet hemocyanine (KLH).

8. Use according to any one of claims 1 to 7, characterised in that the DCs are additionally charged with an adjuvant, especially with tetanus toxoid.

9. Use according to any one of claims 1 to 8, characterised in that the DCs have been generated in vitro from peripheral blood mononuclear cells (PBMCs).

10. Composition for triggering IL-12 release from DCs containing LPS, IFN-γ and a tumor antigen.

11. Composition according to claim 10, characterised in that it

is calf-serum free.

12. Use of a combination of LPS, IFN-γ and a tumor antigen for triggering IL-12 release from DCs.

13. Use according to claim 12, characterised in that the DCs have been loaded with an antigen from a tumor cell from a patient having said tumor.

14. Kit for triggering IL-12 release from DCs comprising

- LPS,
- IFN-γ and
- a tumor antigen.

15. Use of a kit according to claim 14 for triggering IL-12 release from DCs.

16. Use according to claim 15, characterised in that the DCs have been loaded with an antigen from a tumor cell from a patient having said tumor.